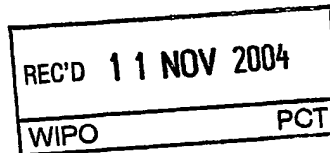




GB04/4173



INVESTOR IN PEOPLE

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Andrew Gersey

Dated 3 November 2004

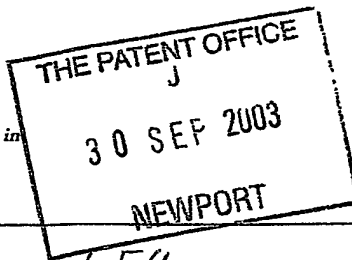
BEST AVAILABLE COPY

BEST AVAILABLE COPY



Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)



The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

2. Patent application number
(The Patent Office will fill this part in)

0322791.5

30 SEP 2003

3. Full name, address and postcode of the or of each applicant (underline all surnames)

LIFEFORCE GROUP PLC
UNIT 35
IMPERIAL HOUSE
IMPERIAL SCIENCE PARK
NEWPORT
GWENT
NP10 8UH
WALES

Patents ADP number (if you know it) 86583 20001

If the applicant is a corporate body, give the country/state of its incorporation

1. Title of the invention

BLOOD CO-PROCESSING FOR CONTINGENT
AUTOLOGOUS LEUKOCYTE TRANSPLANTATION

5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

DR. VINCENT A. PRICE
CARR HOUSE
KIRKLEES HALL
CLIFTON
WEST YORKSHIRE

Patents ADP number (if you know it) 8337248001 HD6 4HD

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months.

Country

Priority application number
(if you know it)

Date of filing
(day / month / year)

7. Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute (see note f)

Number of earlier UK application

Date of filing
(day / month / year)

8. Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?

Answer YES if:

- a) any applicant named in part 3 is not an inventor, or
- b) there is an inventor who is not named as an applicant, or
- c) any named applicant is a corporate body.

Otherwise answer NO (See note d)

YES

Patents Form 1/77

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form

Description 33

Claim(s) 7

Abstract

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77) 1

Request for a preliminary examination and search (Patents Form 9/77) 1


Request for a substantive examination (Patents Form 10/77)

Any other documents (please specify)

LETTER REQUESTING ACCELERATED SEARCH
AGREEMENT DATED 26/9/03, CHEQUE

11. I/We request the grant of a patent on the basis of this application.

Signature(s)



Date 26/9/03

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

01484 710 970

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered YES in part 8, a Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- Part 7 should only be completed when a divisional application is being made under section 15(4), or when an application is being made under section 8(3), 12(6) or 37(4) following an entitlement dispute. By completing part 7 you are requesting that this application takes the same filing date as an earlier UK application. If you want the new application to have the same priority date(s) as the earlier UK application, you should also complete part 6 with the priority details.

**BLOOD CO-PROCESSING FOR CONTINGENT AUTOLOGOUS
LEUKOCYTE TRANSPLANTATION**

5

Field of the Invention

The invention relates to a process for producing a leukocyte bank, to leukocyte cell banks and preserved leukocyte compositions created thereby and to various forms of therapy based thereon.

10

Background to the Invention

Cell banks

15 Cell banking is a service industry in which live cells are stored for later use. It has been practised for decades, and is exemplified by the storage of bovine sperm cells for the artificial insemination of cows.

20 With the technical advances that are being made in bio-medical research and tissue engineering, it is being recognized that many possibilities may exist for the use of human stem cells for various replacement therapies. These developments have led to a growing demand for facilities where stem cells of individuals can be isolated, cryo-preserved, and stored for later (autologous) use. For example, the desirability of storing the cord blood stem cells of newborns is becoming
25 increasingly recognized and as a result there is a rapidly increasing number of deposits of such stem cells in private cell banks.

30 With this growth in interest in cell and tissue banking has come an increasing awareness of the practical problems. It has become clear that cell banks intended to provide a long-term cellular resource are vulnerable to random events that lead to loss of viability of some or all of the deposits and that the risks associated with such events increase with the size of the bank and with the duration of storage. Deposit integrity is also crucially important: the way in which the deposits are prepared, stored, handled and used may crucially determine the integrity of the

bank: this is particularly important when cross-contamination of deposits can lead to the spread of disease or to inappropriate or dangerous physiological consequences (such as may arise from the administration of allogeneous cellular material when autologous grafting is indicated). With large banks, information storage, processing and deposit cataloguing are also extremely important.

Such issues have led to a growing number of statutory provisions and codes of practice governing the production, maintenance and use of cell banks in most countries: in the United Kingdom, cell banking is now controlled by a comprehensive regulatory framework.

Contingent autologous transplantation (CAT) therapy

A form of therapy has recently been described (see WO 00/29551 and WO 01/88099) in which various tissues (including leukocytes) are removed from a healthy donor and stored in a tissue or cell bank for later autologous transplantation in the event that a need for such autotransplantation arises at some future date. This form of therapy is herein referred to as *contingent autologous transplantation* (CAT) therapy.

For any given tissue or cell type, the need for CAT therapy is likely to arise in only a fraction of the healthy population. As a result, the effectiveness of CAT therapy depends crucially on the generation of comprehensive cell and tissue banks in which deposits from a large percentage of the population are included.

Accordingly, it has been proposed that CAT therapy be facilitated by the construction of comprehensive tissue banks. However, the nature of CAT therapy places unique and stringent demands on any such tissue bank. In particular, CAT therapy implies a large number of participating donors (and consequently a large number of deposits), relatively long-term storage, good retention of tissue function over time and great flexibility in ultimate therapeutic use.

Such problems are particularly acute in the case of leukocyte cell banks, where the absolute number of cells available is relatively small, the ultimate therapeutic

efficacy may depend critically on the function of a small subset of cells and the activity profile of the stored leukocytes may change over time as the various subsets of cells respond to storage in different ways. To date, no leukocyte cell banks suitable for CAT have been constructed.

5

Isolation of leukocytes for CAT therapy by leukapheresis

Leukapheresis is a specific form of apheresis which involves the selective separation and removal of leucocytes from withdrawn blood, the remainder of the
10 blood then being retransfused into the donor. During leukapheresis, the removed blood is passed through a cell separation device which separates nucleated white blood cells from red blood cells and plasma outside the body. The red blood cells and plasma are returned to the individual, as part of the separation process. The process is continuous with blood being removed and returned almost
15 simultaneously after various extractions have been performed.

Leukapheresis therefore makes it possible to remove and return the entire blood volume of the individual several times over and separate out and keep large numbers of white cells without detriment to the individual. The technique
20 therefore relies on the establishment of a vein-to-vein extracorporeal blood circulation and extraction of leukocytes from the recirculating blood.

Leukaphereses are generally automated, and conducted using either continuous or interrupted flow centrifugation or filtration techniques, as described in
25 "Leukapheresis and Granulocyte Transfusions", published by American Association of Blood Banks, Washington DC (1975).

Apparatus for carrying out centrifugation leukapheresis is described in US 3489145 and US 3655123, while that for carrying out filtration leukapheresis is
30 described in US 3802432 and US 3892236. Gravity leukapheresis, in which the forces for both separating and collecting leukocytes are provided by gravity alone, is described in US 4111199.

Many different types of automated leukapheresis apparatus are now commercially available including the Fenwal CS-3000 (Baxter Healthcare, Chicago, IL), the Cobe 2997 (Cobe BCT, Lakewood, CO), the Cobe Spectra, the Cobe 2991, and the Haemonetics V50 (Haemonetics Corp., Braintree, MA). Any of these systems
5 can be used in the processes of the invention, but preferred is the Cobe® system (Cobe BCT, Lakewood, CO, USA), which is capable of extracting between 40% and 50% of the total white cells in the whole blood that passes through the separator, and which can achieve a flow rate of 40-60 ml or more per minute.

10 Leukapheresis has recently been proposed as a means for creating lymphocyte cell banks (see WO 00/29551 and WO 01/88099). However, in these teachings the leukapheresis is employed in order to exploit the possibility of removing, extracting and then returning very large (or even the entire) blood volumes in order to recover large numbers of leukocytes. Thus, once the leukocytes are
15 removed, these teachings indicate that the remainder of the blood should be reinfused into the donor.

In contrast, the present inventors have for the first time recognized that leukapheresis can be used to isolate leukocytes from blood whilst producing one
20 or more further blood fractions as coproduct(s). The coproduct(s) can then be stored separately in a different cell bank or else used for various therapeutic applications. For example, a red blood cell fraction can be stored chilled for up to 35 days, and can be used for treating anaemic patients, victims of trauma and patients undergoing surgery. Plasma can be frozen below -30°C for up to one
25 year, and can be used to reverse anticoagulant treatment and as a component in massive transfusions. Thrombocytes, stored at 20°C and continually agitated to prevent clotting, can be used to prevent bleeding in leukaemic patients or those undergoing chemotherapy or massive blood transfusions.

30 The coprocessing method of the invention can be carried out using traditional leukapheresis (with the donor in-line), provided that the total volume of blood processed and fractionated is low enough to be tolerated by the donor.

Alternatively, the leukapheresis equipment can be applied to an isolated blood sample to isolate and collect not only leukocytes, but also one or more further blood fractions as coproduct(s). In this case, the invention employs a process of *isolated leukapheresis* (as defined herein) for the production of isolated leukocytes together with one or more blood fractions as co-product(s).

Summary of the Invention

Accordingly, in a first aspect the present invention provides a process for producing a leukocyte bank for CAT therapy comprising the steps of:

- (a) providing a blood sample from a healthy donor individual;
- (b) selectively separating and collecting leukocytes from the sample; and
- (c) selectively separating and collecting red blood cells and/or platelets and/or plasma from the sample; and
- (d) cryogenically preserving the leukocytes (and optionally the red blood cells and/or platelets and/or plasma);

wherein steps (b) and (c) are conducted with an automated leukapheresis device comprising a separation device (e.g. a centrifuge rotor or filter), a leukapheresis tubing set and one or more pumps for conveying the sample through the tubing set and the separated leukocytes into a collection vessel.

The leukocytes are preferably first separated and then collected in a holding vessel. The holding vessel serves to hold the leukocytes during the isolation and collection steps (which may take several minutes or even hours to complete) and permits stringent control over the mixing and preserving steps. In particular, the use of a separate holding vessel permits control over the incubation time and concentration of the leukocytes in the cryogenic preservation medium prior to freezing.

The present inventors have found that this precaution significantly improves the retention of leukocyte functionality after subsequent cryogenic preservation and revitalization and helps create a reliable leukocyte bank in which the viability of a

subset of leukocytes in a relatively small number of autologous deposits may ultimately prove to be critical to successful CAT treatment.

Two or more aliquots may be transferred from the holding vessel to two or more
5 independent storage vessels, when each of the independent storage vessels may advantageously comprise a cryopreservation medium in fluid communication therewith.

The term *independent*, as applied to the storage vessels of the invention, is
10 intended to define vessels which can be independently stored (as defined herein). Thus, the independent storage vessels of the invention may have non-contiguous internal volumes. may not share a barrier and may be entirely separate (or separable) vessels.

15 The storage vessels may contain, or be in fluid communication with, a cryopreservation medium. In preferred embodiments, the cryopreservation medium is simply present in liquid form within the storage vessel. In such embodiments, the cryopreservation medium may be isolated from the holding vessel(s) by any convenient means, for example by: (a) a valve; (b) a breakable
20 seal, (c) gravity in conjunction with the spatial arrangement of the holding and storage vessels; and/or (d) surface tension within a tube connecting the holding and storage vessels.

In other embodiments, the cryopreservation medium is in fluid communication
25 with the storage vessel but not contained therein, for example being held in a satellite cryogenic medium vessel. In such embodiments, the satellite cryogenic medium vessel may be reversibly isolated from the storage vessel by any convenient means. for example by: (a) a valve; (b) a breakable seal, (c) gravity in conjunction with the spatial arrangement of the satellite and storage vessels;
30 and/or (d) surface tension within a tube connecting the satellite and storage vessels.

The storage vessels may be double containment vessels. The term *double containment*, as used herein in relation to the storage vessels of the invention, is a

term of art defining a container having at least two barriers: a proximal barrier, in contact with the separated leukocytes, which contains the leukocyte sample and isolates it from the outside environment, and a secondary barrier which acts as a fail-safe to isolate the leukocyte sample from the outside environment in
5 circumstances where the proximal barrier fails (for example after accidental physical or chemical trauma).

Preferably, the process further comprises the step of mixing each aliquot of the separated leukocytes with a cryopreservation medium within each storage vessel.
10

The process may also comprise retrievably depositing the preserved leukocytes into two or more independent storage systems to produce a leukocyte bank which exhibits deposit redundancy. As a further precaution, two or more aliquots are used according to the invention in order to provide for deposit redundancy.

15 Preferably, three, four, five or greater than five separate aliquots are used according to the invention.

The storage vessels are preferably detachable from the holding vessel and from any ancillary tubing and/or satellite vessels, for example by releasable clamps, releasable docking components, tearable seals, heat crimping or by use of so-called "docking systems" for sterile sealing.
20

The independent storage systems into which the aliquots are retrievably deposited are independent in the sense that they do not share a determinant of viability selected from: (a) power supply and/or (b) site or location. For example, in cases
25 where the storage systems depend on a supply of electricity for their continued cryopreservation, then the electricity supplies must not originate from a single generator or supplier.

30 Preferably, each independent storage system is sited to be geographically remote from its counterpart(s), so lessening the chances of coincidental destruction or damage by natural or man-made disasters (such as fire, flood or contamination).

The separation and collection steps are preferably conducted within a closed or functionally closed system and may be applied iteratively to a series of blood samples from different healthy donor individuals.

5 As used herein, the term *closed system*, in the context of the process of the invention, is used to define blood processing apparatus consisting of elements which are sterile and isolated from the outside environment by aseptic barrier(s) and in which all components are fully integral, being attached and/or assembled at the manufacturing site.

10

As used herein the term *functionally closed system*, in the context of the process of the invention, is used to define blood processing apparatus consisting of elements (e.g. tubing sets) which are assembled at the device manufacturing site and which use sterile barrier filters (e.g. 0.22 micron filters) or so-called "docking systems" for the sterile interconnection by the end user to generate a wide variety of arrays of tubing, channels, filters, satellite bags and other vessels.

15

The process may further comprise the step of digitally storing information obtained from each donor individual in a digital information unit so as to permit matching of leukocyte deposit and donor for later autologous transplantation.

20

The cryogenic preservation step conveniently comprises freezing to a temperature at or below about -160°C, which can be achieved using liquid nitrogen. If longer periods of storage and/or enhanced retention of functionality are required then freezing to a temperature at or below about -269°C may be effected using liquid helium.

25

Any of a wide range of suitable cryopreservation media may be used according to the invention, but preferred are media comprising a suitable penetrating cryoprotectant. Particularly suitable for use as a penetrating cryoprotectant is DMSO, which may be used for example at a concentration of up to 10%.

30

The cryopreservation medium may further comprise an anticoagulant (such as acid citrate dextrose, EDTA, heparin or mixtures thereof), a nuclease (for example

a Dnase and/or Rnase as well as a physiologically acceptable medium (for example, phosphate buffered saline). The cryopreservation medium may also further comprise a proteinaceous composition, such as blood serum or a blood serum component and/or a polysaccharide (which may be particularly preferred in
5 embodiments where plunge freezing is employed).

Particularly preferred proteinaceous compositions for use in the cryogenic preservation media of the invention comprise blood albumin (e.g. bovine serum albumin or human serum albumin). Particularly convenient is the use of human
10 blood serum isolated from the blood sample of the donor individual. This can be isolated as a co-product together with the leukocytes.

Any donor may be used as a source of blood sample in the processes of the invention, provided that the donor is healthy, as herein defined. However, the
15 invention finds particular application in relation to donor individuals which are predisposed to a leukocyte deficiency, are not in remission from a leukocyte deficiency, are juvenile, adolescent or adult, are at risk of developing a leukocyte deficiency, are human individuals between the ages of about 12 to 30 (e.g. 15 to 25) and/or have a fully-developed immune system.

20 The blood sample for use in the process of the invention may be one which is in fluid communication with the donor individual (for example, in circumstances where leukapheresis is used to selectively separate the leukocytes). Preferably, however, the sample is an isolated blood sample (as defined herein) and is
25 produced by a process of *isolated leukapheresis* (as defined herein).

The leukocytes separated in step (b) may comprise (or consist essentially of) granulocytes, lymphocytes and/or monocytes. In many embodiments of the invention, no steps are taken to enrich for (or isolate) any particular class (or
30 classes), subpopulations or cell types.

However, in some embodiments the separation step (b) comprises the selective separation of a particular class or type of leukocyte. This may be done in

circumstances where it is desired to effect the selective separation of B-cells and/or T-cells and/or dendritic cells (or mixtures thereof).

The information stored in step (i) comprises at least that necessary to permit
5 matching of deposit with donor, in order that later autologous transplantation can be carried out. Preferably, the information comprises genetic information, the date at which the blood sample was collected from the donor individual, the age and sex of the donor individual, the clinical status of the donor individual, the medical history of the donor individual, biographical data identifying the donor
10 individual, details of the processing and storage conditions used to prepare the deposit as well as data identifying the person(s) responsible for processing the sample(s).

If genetic information is stored, then this preferably comprises sequence
15 information relating to one or more gene(s), single nucleotide polymorphism (SNP) data and/or one or more genetic fingerprint(s).

Any suitable digital information unit may be used to store the information. Preferably, this takes the form of at least one digital computer comprising a
20 database. The database may carry data on a carrier of any convenient form. Preferably, the information is stored independently on two or more carriers so that the database exhibits redundancy. This protects against data loss in the event of failure, corruption or loss of one of the computers or data carriers.

25 Preferably, the process further comprises the step of labelling the storage vessels with information sufficient to permit matching of the leukocyte deposit and donor. For example, the storage vessels may be labelled with information:

- (a) describing the contents of the vessel (for example, sample size, number and/or volume); and/or
- 30 (b) identifying the leukocyte bank; and/or
- (c) recording the date at which the blood sample was collected from the donor individual; and/or
- (d) comprising a statement that each package is for single patient use only; and/or

- (e) comprising instructions for opening, aseptic presentation and further storage.

Any convenient form of labelling may be used. Thus, the labelling may comprise the physical attachment of an information carrier (e.g. a bar code) to the storage vessels themselves. Alternatively, the labelling may be effected by the non-physical association of the vessels with the information carrier (for example, *via* the correlation between the physical geometry or organization of the deposits in the bank and the entries in the database).

10

The invention also contemplates treatment of the leukocytes, for example including any or all of the following: *in vivo* prior to provision of the blood sample, *in vitro* prior to separation step (b), *in vitro* after separation step (b) but prior to preservation step (f) and/or *in vitro* after preservation step (f).

15

Any suitable method for selectively separating the leukocytes from the sample may be used. In preferred embodiments, the leukocytes are selectively separated in step (b) using leukapheresis apparatus (as described in more detail herein).

20 The leukapheresis apparatus preferably comprises: (a) a separation device (e.g. a centrifuge rotor or filter); (b) a leukapheresis tubing set; and (c) one or more pumps for conveying the sample through the tubing set and the separated leukocytes into the collection vessels.

25 Particularly preferred is the separation and collection of leukocytes in steps (b) and (c) using automated leukapheresis apparatus.

Particularly preferred is the use of automated leukapheresis equipment in isolated leukapheresis mode (as defined herein), or in the more usual mode of automated continuous or interrupted flow centrifugation leukapheresis or continuous or interrupted flow filtration leukapheresis.

30

In another aspect, the invention contemplates a process for producing a leukocyte composition for autotransplantation into a donor individual comprising the steps of:

- (a) producing a leukocyte cell bank by the process of the invention;
- 5 (b) matching the donor individual with a leukocyte deposit to identify an autologous leukocyte deposit (e.g. using the information described above);
- (c) retrieving a storage vessel containing an aliquot of preserved autologous leukocytes;
- (d) revitalizing the preserved autologous leukocytes to produce a leukocyte
- 10 composition for autotransplantation into the donor individual.

The invention also contemplates a leukocyte composition and a leukocyte cell bank obtainable (or obtained) by the process of the invention.

- 15 Also contemplated are various therapeutic uses for the processes, compositions and banks of the invention. Accordingly, the invention contemplates the leukocyte composition of the invention for use in therapy, for example in autotransplantation (e.g. in restorative or remedial autotransplantation).

20

Detailed Description of the Invention

I. Definitions

- 25 Where used herein and unless specifically indicated otherwise, the following terms are intended to have the following meanings in addition to any broader (or narrower) meanings the terms might enjoy in the art:

- 30 The term *leukapheresis* is a term of art used herein to define a procedure involving the selective separation and removal of leukocytes from the withdrawn blood of a donor, the remainder of the blood then being retransfused into the donor.

A *leukapheresis device* is a term of art defining any device capable of performing leukapheresis, irrespective of the means employed in the device to separate and remove the leukocytes.

- 5 The term *isolated leukapheresis* is used herein to define a novel form of leukapheresis which is performed on an *isolated* blood sample.

The term *isolated blood sample* is used herein to define a blood sample which is not in fluid communication with the blood of the donor from which it originated.
 10 Thus, in the process of isolated leukapheresis which is applied to isolated blood samples, the leukapheresis device is not in fluid communication with the individual providing the blood sample and/or the remainder of the blood in the sample is not retransfused into the individual.

- 15 The term *autotransplantation* is used herein to define autologous transplantation (autogeneic or self-to-self transplantation), wherein the term *autologous* is used to indicate that the transplantation is to the same organism (i.e. the same individual) from which the cellular material (e.g. leukocytes) was removed. As used herein, *transplantation* defines any procedure involving the introduction of cellular
 20 material (e.g. leukocytes) into an organism, and so any form of transplantation or grafting known in the art is encompassed.

The term *dormancy* is used herein to define any state of suspended animation or stasis, and procedures for achieving this are well known in the art, as described
 25 below. Any of the known procedures may be used, including cryopreservation. Thus, the leukocytes may be held or maintained in a quiescent, inactive or non-proliferating state.

The term *healthy* is used herein in relation to an individual donor to indicate that
 30 the individual is not suffering from a leukocytic deficiency (as herein defined). Thus, the term *healthy* as used herein encompasses *non-diseased* individual donors in a state in which the individual donor is not suffering from any disease or disorder, or is not manifesting any symptoms of said disease or disorder (i.e. is asymptomatic or is in a pre-clinical condition). In particular, term *healthy* as used

herein encompasses individual donors not suffering from, or demonstrating symptoms of, the disease or disorder which it is subsequently intended to treat by the autotransplantation procedure.

5

II. Blood samples

The invention may be applied to any form of blood sample, provided that the sample comprises at least some leukocytes from the individual donor.

10

The blood sample may be subjected to various treatments *ex vivo* prior to use in the process of the invention. Typically, for example, the blood sample is chilled prior to use. Other treatments may include the addition of preservatives and/or anticoagulants.

15

The blood sample may also be treated *in vivo* prior to collection by administering various agents to the donor individual before or during sample collection.

20

Examples of treatments (which may be applied *ex vivo* and/or *in vivo*) are discussed in more detail in the section entitled "Leukocyte treatments", below.

25

It is generally preferable to sample at least 450-500 ml of blood from the individual donor, which is the equivalent of a unit of blood as provided by a blood donor for the UK blood transfusion service. If possible a number of samples (e.g. several 450-500 ml samples) are taken over a period of time (e.g. over 2-3 weeks, preferably 2-3 months or over 6 months or a year, 2 or 3 years or more). One or more of these can then be divided or combined into a number of leukocyte cell bank deposits. The removal of a unit of blood is commonplace with over three million units of blood being taken, for allografting, from individuals annually in the UK alone.

30

The blood removed is soon replaced and, therefore, multiple samplings of a unit of blood from an individual can be provided over a year, say 2-12 unit samplings if necessary, without detriment to the individual being sampled.

III. Selection of donor individuals

5 General considerations

Restorative autotransplantation is a form of therapy that might ultimately be indicated for any individual. Consequently, the invention may be usefully applied to the generation of comprehensive leukocyte cell banks covering as large a
10 number of different individuals as possible in order that restorative autotransplantation can be carried out in any of the represented individuals should the need arise.

It is therefore contemplated that the invention be applied as broadly as possible so
15 that a comprehensive leukocyte cell bank can be assembled. However, since the quality of the individual deposits will depend (at least to some extent) on the health status of the individual donor at the time of blood sample donation, it is preferred that the blood sample for use in the processes of the invention be taken from healthy individual donors.

20 Other factors also affect donor selection: for example, the blood sample for use in the processes of the invention may advantageously be obtained from individual donors when they are young, preferably in adolescence or early adulthood. In the case of humans, blood sampling (preferably multiple sampling) at the ages of
25 about 12 to 30, preferably 15 to 25 is preferred. Especially preferably, sampling is from the age of 16 or 17 upwards, for example in the age range 16 to 30, 17 to 30, or 18 to 30, or perhaps 18 to 35 or 40. It is thus preferred that the cells be obtained when the host organism is mature, or reaching maturity, but before the processes of ageing or senescence have significantly set in. In particular, it is
30 preferred and advantageous that the immune system of the host organism is mature or fully developed.

However, the obtention of cells outside these ranges is encompassed, and cells may be obtained at any post-natal life stage e.g. from juvenile host organisms e.g.

in mid-to late childhood, or even infants, or from older individuals.

Sampling from post-natal or older hosts allows multiple samples to be collected, thereby increasing the opportunity of storing sufficient number of cells. In addition sampling from juvenile or older hosts overcomes the ethical requirements such as providing informed consent.

Sampling from adolescent or adult host organisms is preferred since the sampled cells, from blood in particular, will contain a greater proportion of valuable mature T-cells capable of recognising aberrant cell populations, such as cancer cells or virally infected cells. Thus, when blood samples are used, it is advantageous that they are taken from an individual with a mature immune system (i.e. not foetal or neonatal).

Thus, the invention contemplates the use of blood samples collected from donor individuals at a stage when there is no direct prediction, suggestion, or suspicion that a particular disorder or disease may develop, for use against a future possible or unpredicted event, or an event which may occur simply by chance, rather than an anticipated or suspected or predicted illness or condition. Thus, in certain embodiments of the invention, the donor individual is not predisposed to, or at risk from, any particular disease or disorder e.g. not exhibiting any symptoms or manifestations predictive of a subsequent disease or disorder. Likewise, the host organism is preferably not suffering from any injuries or damage which may give rise to an anticipated or expected condition.

Indeed, for certain applications (for example, the generation of leukocyte cell banks for subsequent restorative autotransplantation) it is preferred that the blood sample for use in the invention be obtained from the donor individual before any disease or disorder develops or manifests itself, and more preferably when the host organism is in general good health, and preferably not immunocompromised in any way. In such embodiments it is particularly advantageous to sample the blood from donor individuals at a time when the organism has not previously exhibited symptoms of or presented with or been diagnosed as suffering from the disease or disorder which is subsequently to be treated, i.e. when the host

organism is healthy and not "in remission" e.g. not in a state of partial or full recovery from the leukocyte deficiency to be treated.

Predisposed donor individuals

5

Advances in therapy continue to be made, and our greater understanding of disease processes helps us to modify and refocus our therapeutic approaches to alleviate disease and suffering. Such understanding has been greatly advanced by technological improvements in the field of molecular biology. We are now in a position to follow the pathogenesis of diseases at a molecular level, and recognize the importance of an individual's genetic make-up in predisposing them to certain diseases. For example, we are aware that some individuals, because of their genetic composition, are prone to certain diseases.

10

Many of the diseases to which certain individuals can be predisposed are leukocyte deficiencies, which term is used herein to indicate a condition in which the administration of autologous leukocytes is indicated. Such conditions therefore include those in which an individual has acquired a disease, infection or condition involving leukocyte dysfunction or a disease, infection or condition in which the augmentation or stimulation of endogenous leukocyte activity is indicated. Detailed examples of particular leukocyte deficiencies are set out in the section entitled "Exemplary indications", below.

20

Through genetic testing, therefore, it is now possible to identify those individuals predisposed to a leukocyte deficiency (e.g. any of various forms of cancer, immune disorder or infection).

25

Furthermore, our knowledge of the body's immune system, and in particular the way in which it recognises and kills virally infected and tumour cells, continues to advance. We now know that in order to elicit cell-mediated immunity, an offending cell (e. g. a virally infected or tumour cell) must co-present an HLA class I restricted tumour or viral epitope with danger signals such as GM-CSF and/or TNF-alpha. so that the antigen presenting cells (APC) of the immune system will express co-stimulatory signals such as B7 and IL-12 in conjunction

30

with antigen to the interacting cytotoxic T-lymphocyte (CTL) population. The co-presentation leads to the production of clones of both activated and memory cells (for review see Nature Medicine Vaccine Supplement 4 (1998) 525). In the absence of these additional signals, HLA-I antigen-restricted T-cells which
 5 recognise offending cells are processed for destruction or desensitization (a bodily process presumably put into place to avoid the development of e.g. autoimmune disease). The induction of such tolerance is because of either ignorance, anergy or physical deletion (Cold Spring Harbour Symp Quant Biol 2 (1989) 807; Nature 342 (1989) 564; Cell 65 (1991) 305; Nature Med 4 (1998) 525).

10

It is now clear that tumour cells do not automatically co-present danger and/or co-stimulatory signals. Hence, the spawning of a tumour may lead to eradication of the very T cell clones that provide cell-mediated immunity against the tumour. A patient presenting with a cancer, leukaemia/lymphoma or sarcoma etc, therefore,
 15 may have already removed their innate ability to destroy the tumour, by default.

20

However, if the required T lymphocytes, or a sample thereof, were removed from the patient prior to the onset of proliferative disease, the relevant T-cell population could now be returned to the patient, after the necessary co-stimulation of the T-cells, so as to alleviate disease. Co-stimulation may be provided at the same time as the cells are returned to the patient, or after they are returned through further treatment (s) of the patient, or without stimulation other than that naturally produced by the patient. Activation/stimulation of the cells may also initially be induced *in vitro* prior to reinfusion.

25

30

The present invention therefore finds particular application in the case of individuals predisposed to the development of a leukocyte deficiency. It therefore represents a means for sampling leukocytes from a healthy donor individual for subsequent transplantation to that same individual in a subsequent autologous (autogeneic) transplantation procedure, when the need or desire to do so arises. Although the predisposed individual may never receive the cells because no disease to be treated by this method ever occurs, the invention nevertheless may be used to provide some form of insurance against the heightened risk of a

leukocyte deficiency arising in the individual.

Similarly, individuals with no diagnosed predisposition may choose to provide samples for incorporation into the leukocyte cell bank of the invention for
5 prospective use by themselves prior to travelling abroad. Such use might include for the treatment of infections contracted whilst abroad.

In addition, it is well recognized that the ageing process makes individuals more susceptible to disease. The basis for the susceptibility appears to be in the loss of
10 immune function resulting from a significant decrease in T and B cell numbers/activity during ageing (Mech Ageing & Dev 91 (1996) 219; Science 273 (1996) 70; Mech Ageing & Dev 96 (1997) 1). Disease susceptibility is particularly pertinent when elderly patients are subjected to e.g. surgery in a hospital
15 environment, where they are prone to opportunistic infections with serious or even fatal consequences. Blood samples taken from such individuals much earlier in life and processed according to the invention for inclusion in a leukocyte cell bank could provide the opportunity for restorative autotransplantation in such
circumstances.

20 Such an approach could be used more broadly to provide for a method of augmenting the patient's immune system after surgery in order to lessen the likelihood of post-operative complications caused by opportunistic infections. The invention, therefore, could be used as a prophylactic therapy, e.g. for elderly patients when they are more susceptible to disease.

25

IV. Leukocytes

It will be appreciated that the separation and/or removal of leukocytes from the
30 blood sample during leukapheresis need not be absolute. Rather, the removal and/or separation of a fraction of the total leukocytes present in the sample is sufficient in most circumstances. Those skilled in the art will readily be able to determine the appropriate size of the fraction to be removed, which will vary *inter alia* according to the use to which the isolated leukocytes are to be put, the size of

the sample, the status of the donor, the nature of the leukocytes and the particular leukapheresis device employed.

5 The leukocytes collected in the processes of the invention are to some degree isolated from the original blood sample. The term *isolated* is used here to indicate that the isolated leukocytes exist in a physical milieu distinct from that in which they occur *in vivo* and does not imply any particular degree of purity. Indeed, the absolute level of purity is not critical, and those skilled in the art can readily determine appropriate levels of purity according to the use to which the leukocytes
10 are to be put.

The separation and collection of the leukocytes in the processes of the invention also does not necessarily imply that any particular class or type of leukocyte is preferentially separated and collected. Rather, the leukocytes of the invention
15 include any white blood cell, including granulocytes, lymphocytes and monocytes.

Granulocytes include myelocytes, basophils, eosinophils and neutrophils. Lymphocytes include B, T lymphocytes and natural killer cells. Monocytes include mononuclear phagocytes and other macrophages.
20

However, in some embodiments the leukocytes which are separated and collected preferably comprise one or more specific leukocyte cell types. A preferred cell type is the lymphocyte, especially a T-lymphocyte (T-cell). Mature T-lymphocytes are particularly preferred.
25

Since the total mature T-cell number per litre of blood ranges between $1-2.5 \times 10^9$ for humans, a 100 ml sample of blood typically contains $1-2.5 \times 10^8$ mature T-cells and this is generally sufficient to provide an adequate representation of the entire mature human T-cell population for the beneficial effect. However,
30 depending on the fraction of total leukocytes separated and collected by the leukapheresis device and the efficiency of any revitalizing technique employed, preferably at least 100 ml, 115 ml, 200 ml or 300 ml and even more preferably in excess of 400 or 500 ml of blood sample is used in order to obtain the appropriate

number of mature T-cells to support a beneficial therapeutic effect for return to the individual if and when they become ill.

Standard techniques are known in the art which permit selection of particular subpopulations of lymphocytes from a sample comprising a mixed population of lymphocytes. Examples of such subpopulations are CD3⁺, CD8⁺, CD4⁺ and CD16/56⁺ (natural killer) T cells and CD19⁺ B cells. For example, any one or any mixture or combination of such subpopulations of T cells can be used in the methods, uses and compositions of the invention, and they are readily obtained by means of well known methods such as FACS (Fluorescence Activated Cell Sorting) and haemocytometry systems.

V. Leukocyte treatments

The leukocytes may be subjected to various treatments. Such treatments may, for example, result in expansion of some or all of the representative cell subsets, improve the long-term viability of the leukocytes during the dormancy period, improve their therapeutic potency, remedy a deficiency or defect exhibited by some or all of the leukocytes (as is the case, for example, in remedial autotransplantation therapeutic modalities) and/or render their subsequent use in autotransplantation safer.

The treatments can be carried out before or after the leukocytes are rendered dormant (and before or after autotransplantation is carried out). Moreover, the treatments may be applied after the blood sample is taken (i.e. be carried out *ex vivo*) either prior to rendering the cells dormant or after revitalization. For example, treatment of the leukocytes may be effected by co administration of a separate composition, sequentially or simultaneously with the leukocyte composition, during autotransplantation. Treatment of the leukocytes can be effected immediately prior to autotransplantation.

Alternatively (or in addition) the treatments may be applied to the leukocytes while still *in vivo* prior to blood sampling by the administration of e.g. growth factors or cytokines (see below).

5 Exemplary pre-transplantation treatments may include various genetic modifications, such as the incorporation of a negative selection marker (as described, for example, in W096/14401, the content of which is incorporated herein by reference). Such treatment permits ablation of the leukocytes after transplantation or titration of dose versus response. Other genetic interventions
10 may include regulating or modifying the expression of one or more genes (e. g. increasing or decreasing gene expression), inactivating one or more genes, gene replacement and/or the expression of one or more heterologous genes). Other genetic modifications include the targeting of particular T-cells (as described in W096/15238, the content of which is incorporated herein by reference), and the
15 modification of the T-cell receptor repertoire/expression with antibodies to make T-cell chimaeras.

Other treatments contemplated by the invention include the exposure of the leukocytes with one or more stimulatory molecules, for example antigens (e.g.
20 cancer or viral antigens), antibodies, T cell recognition epitopes, peptides, blood factors, hormones, growth factors or cytokines or combinations thereof.

For example, the leukocytes may be treated *in vitro* (or *in vivo* prior to blood sampling) with antigens (for example cancer (e.g. prostate-specific antigen 1 or
25 prostate-specific antigen 2, her-2/new, MAGE-1, p53, Ha-ras and c-myc) or viral antigens), antibodies, T cell recognition epitopes, peptides, blood factors, hormones, growth factors or cytokines or combinations thereof. The stimulatory molecules may be synthetic, recombinant or may be purified or isolated from the human or animal body. Particularly useful in this respect are stimulatory
30 molecules selected from IFN-alpha, IFN-beta, IFN-gamma, Il-1a, Il-1b, Il-2, Il-3, Il-4, Il-5, Il-6, Il-7, Il-8, Il-9, Il-10, Il-11, Il-12, Il-13, Il-14, Il-15, GM-CSF, M-CSF, G-CSF, LT and combinations of two or more of the foregoing. Such treatments may modify the growth and/or activity and/or state of differentiation of

the leukocytes, and/or may serve to separate or selectively isolate or enrich desired leukocyte cell types or to purge unwanted cells.

Recent advances have been made in the way cells may be obtained for subsequent autotransplantation. For example, investigations into the agents which regulate haematopoiesis have led to the isolation of a series of factors that influence the proliferation and differentiation of lymphocytes. These agents include the cytokines (such as the interleukin series IL-1 to IL-18 and the leukotrienes) and growth factors such as the TNF's, the TGF's, FGF's, EGF's, GM-CSF, G-CSF and others. A number of these factors are now available commercially for clinical use, and some have been shown to increase substantially the number of lymphocytic cells and, in particular, immature T-lymphocytes in the peripheral blood. Their administration to the donor individual prior to blood sampling permits the quantity and/or quality (in terms of the number and nature of leukocyte subtypes present) to be controlled and makes it possible to recover large quantities of the cells of interest, e.g. immature T-lymphocytes, directly from the donor individuals peripheral blood sample without the need to sample the marrow.

Other pre-transplantation treatments include culture of the leukocytes (or a sub-population thereof). For example, the leukocytes may be cultured to increase cell numbers. For example, the cells may be passaged, according to methods well known in the art. Such culturing may be carried out before or after the leukocytes are rendered dormant, or both before and after dormancy is induced.

Thus, in the case where the leukocytes include T-cells, the T-cells may be co-stimulated prior to transplantation and/or exposed to tumour antigens (optionally together with co-stimulatory factors) prior to autotransplantation.

30

VI. Leukapheresis devices

Many different types of automated leukapheresis devices are presently commercially available. Such devices usually comprise at least three separate elements: (1) a *separation device* (e.g. comprising a membrane or centrifuge

rotor. which provides the forces for separating the leukocytes from the various other blood components; (2) one or more *pumps* for conveying the blood sample to the separation device. for removing the separated leukocytes and for maintaining the forces necessary for transfusion and retransfusion, and (3) a
5 (normally disposable) *tubing set* which holds the blood and its various fractions in a particular geometry within the separation device, defines fixed channels through which the blood flows (normally in a circuit from the donor, through the leukapheresis device and back to the donor) as well as vessels (usually bags) for the collection of the separated leukocytes and/or other blood fractions or fluids.

10

Any of a wide variety of commercially available leukapheresis devices may be used according to the present invention. The particular way in which the leukapheresis device is operated will depend on a number of factors, including the nature of the separation device (e.g. centrifuge, filter etc.), the type of leukocyte
15 sample required, the volume of the blood sample to be processed, the identity and status of the donor individual, the ultimate use to which the leukocyte composition is to be put and the nature of any treatments applied to the blood sample prior to processing according to the invention. Thus, those skilled in the art will readily be able to establish the appropriate operational parameters.

20

Preferably, however, an automated leukapheresis device is selected to minimize the need for operator intervention and/or training. Commercially available leukapheresis systems vary in the time and/or expertise required of an individual to prepare and operate it. For instance, reducing the time required by the operator
25 to load and unload the tube set, as well as the complexity of these actions, can increase productivity and/or reduce the potential for operator error. Moreover, reducing the dependency of the system on the operator may lead to reductions in operator errors and/or to reductions in the credentials desired/required for the operators of these systems.

30

Performance-related factors are also relevant, and may be judged *inter alia* in terms of the "collection efficiency" of the leukapheresis system. The "collection efficiency" of a system may of course be gauged in a variety of ways, such as by the size of the fraction of leukocytes collected in relation to the total leukocytes

present in the sample. Performance may also be evaluated based upon the effect which the leukapheresis procedure has on the various blood component types. For instance, it is desirable to minimize the adverse effects on at least the leukocytes of the apheresis procedure. It may also be desirable to reduce platelet activation, in order to avoid degeneration in sample quality during processing.

Particularly preferred is the Cobe® system (Cobe BCT, Lakewood, CO, USA).

VII. Cryogenic preservation

Any suitable means may be employed for cryogenic preservation.

According to a preferred cryopreservation procedure, the cells are frozen preferably to a temperature below -160°C . A particularly preferred means of achieving dormancy is to freeze the cells to the boiling point of helium (He), i.e. to about -269°C or below.

As described in Freshney's (Freshney's Tissue Culture of Animal Cells (Culture of Animal Cells: A Manual of Basic Technique, Wiley Liss, 1994)), the cells may be suspended in a suitable medium (e. g. containing up to 10% DMSO) and cooled at a controlled rate (e. g. 1°C per minute to -70°C , then into liquid/gas N_2). Such conventional procedures may be adapted to cool the cells into He/ N_2 mixtures or He. Alternative methods of achieving and/or maintaining cell dormancy include cooling to 4°C .

VIII. Revitalization

Following dormancy, the cells are revitalised prior to use in transplantation. Again, this may be achieved in any convenient manner known in the art, and any method of revitalising or reviving the cells may be used.

Conveniently, this may, for example, be achieved by thawing and/or diluting the

cells. Techniques for revitalisation are well known in the art. Cells may be thawed by gentle agitation of the container holding the cells in water at 37°C, followed by dilution of DMSO to 1% or below, e. g. with medium or serum.

- 5 Cells may be implanted immediately or after recovery in culture. Revitalisation is designed to re-establish the usefulness of the cells e.g. in prophylaxis or curative therapy.

10

IX. Cell banking

The leukocyte compositions of the invention are banked, thereby creating a leukocyte bank.

- 15 Any suitable cell banking system may be employed, provided that the deposits are retrievable for autotransplantation. This implies the use of some form of labelling, but this need not be in the form of a physical appendage to the individual deposits.

Thus, the leukocyte cell bank of the invention may comprise a plurality of cell
20 storage units for storage of leukocyte compositions. The cell banks of the invention may further include a digital information unit for digitally storing information relating to the identity, location and medical history of the donor individual and/or the conditions associated with the particular deposit (for
25 example relating to the date at which the blood sample was collected from the donor individual, the processing conditions and details of any treatments applied to the leucocytes contained in the deposit).

The digital information unit preferably comprises at least one digital computer having sufficient digital storage capacity for storage of the potentially large
30 amounts of information relating to each deposit.

The leukocyte cell bank of the invention preferably further comprises an arrangement for digital data retrieval interfaced with the digital information unit for retrieving selected information stored in the digital information unit. The data

retrieval arrangement may be integrated with the digital computer. Remote access of the digital information *via* the telephone or the internet may also be provided and may permit rapid and convenient access of the information on a global basis.

5

X. Medical applications

The invention finds application in all forms of therapy and prophylaxis in which the administration of (treated or untreated) autologous leukocytes is indicated (i.e. desirable from a therapeutic perspective).

10

For the purposes of the present invention, in such indications a *leukocyte deficiency* is deemed to have arisen.

15

It will therefore be understood that the leukocyte deficiencies in which the invention finds medical application encompass a very broad spectrum of diseases, syndromes, disorders, conditions and infections. For example, it will be appreciated that a leukocyte deficiency, in the special, broad sense defined above, can arise in circumstances where an individual has acquired a disease, syndrome, disorder, condition or infection involving leukocyte dysfunction as well as in circumstances where an individual has acquired a disease, syndrome, disorder, condition or infection in which the endogenous leukocyte component is seemingly normal but in which alteration, augmentation or stimulation of the normal endogenous leukocyte activity is nevertheless indicated/required. In particular, a leukocyte deficiency as herein defined may be deemed to have arisen either as a result of a non-specific loss of T- and or B-cells, or as a result of a loss or deficiency of a particular T- and/or B-cell clonal population.

25

For convenience, such diseases, syndromes, disorders, conditions or infections are collectively defined herein as *leukocytic deficiencies*.

30

The processes of the invention are employed to create a leukocyte composition (e.g. forming part of a leukocyte cell bank) from a blood sample from a healthy individual donor. Thus, the invention is used to create a cellular resource of healthy leukocytic tissue from an individual donor that can be restored to that

donor individual should the individual acquire a leukocytic deficiency at a later date.

In such therapies (referred to herein as *restorative autotransplantation*), the invention exploits the fact that many leukocytic deficiencies occur as part of a temporal sequence of events (which may or may not be causally interrelated), so that the creation of a leukocyte cell bank at a point in time predating onset of the leukocytic deficiency constitutes a therapeutic resource which can later be used restoratively.

10

The concept of restorative autotransplantation described above can be applied to all healthy individuals, irrespective of factors that might serve as indicators of susceptibility to leukocytic deficiency (for example age, medical history, genetic background and lifestyle). However, it does permit the identification of a

15

particular class of individuals for which the processes of the invention may be particularly advantageously applied, as described in more detail in section III (entitled "Selection of donor individuals"). Moreover, since the leukocyte deficiencies as defined above and treatable according to the invention by restorative autotransplantation embrace an enormous variety of known diseases,

20

these are discussed in greater detail in the following section XII (entitled "Exemplary indications").

XI. Exemplary indications

As mentioned in the preceding section, the therapeutic and prophylactic uses of the invention encompass a very broad spectrum of diseases, syndromes, disorders, conditions and infections.

Infections

30

The invention may find application in the treatment of various infections. In this case, the endogenous leukocyte activity may be normal (or responding normally) but its alteration, augmentation or stimulation is nevertheless desirable. In others

(such as HIV infection) the endogenous leukocyte activity is dysfunctional as a direct consequence of infection.

5 Infections which may be treated or prevented according to the invention include bacterial, fungal or viral infections, or infections by any other organism e.g. a protozoan, nematode, insect or other parasite.

A preferred application is the treatment of AIDS as a result of HIV infection. Here, $CD4^+$ cells can be collected from an individual when healthy or non-
 10 infected, and stored for subsequent transplantation into said individual when HIV infection manifests itself or when AIDS develops, or $CD4^+$ cell count falls etc. Such a procedure may be attractive to an individual with a life-style likely to place them at risk from contracting HIV infection.

15 Cancers, leukaemias and sarcomas

The invention may find application in the treatment and prophylaxis of various malignancies: in general, any malignant or pre-malignant condition, proliferative or hyper-proliferative condition or any disease arising or deriving from or
 20 associated with a functional or other disturbance or abnormality in the cells or tissues of the body.

Therapy or prophylaxis of various forms of cancer represents a preferred embodiment of the invention, and the treatment or prophylaxis of any cancerous
 25 cells or tissues of the body is contemplated.

Thus, the invention is not limited to any one type of proliferative disease (e. g. leukaemias, lymphomas, carcinomas or sarcomas), nor is it restricted to specific oncogenes or tumour-suppressor gene epitopes such as ras, prostate-specific
 30 antigen 1 or prostate-specific antigen 2, her-2/new, myc, myb, fos, fas, retinoblastoma, p53 etc. or other tumour cell marker epitopes that are presented in an HLA class I antigen restricted fashion or other such way so as to be identifiable by a leukocyte. All cancers such as leukaemia, lymphoma, breast, stomach, colon, rectal, lung, liver, uterine, testicular, ovarian, prostate and brain tumours such as

gliomas, astrocytomas and neuroblastomas, sarcomas such as rhabdomyosarcomas and fibrosarcomas are included for the therapy or prophylaxis by the present invention.

5 Thus, the present invention finds application in the treatment or prophylaxis of breast cancer, colon cancer, lung cancer and prostate cancer. It also finds application in the treatment or prophylaxis of cancers of the blood and lymphatic systems (including Hodgkin's Disease, leukemias, lymphomas, multiple myeloma, and Waldenström's disease), skin cancers (including malignant melanoma),
 10 cancers of the digestive tract (including head and neck cancers, oesophageal cancer, stomach cancer, cancer of the pancreas, liver cancer, colon and rectal cancer, anal cancer), cancers of the genital and urinary systems (including kidney cancer, bladder cancer, testis cancer, prostate cancer), cancers in women (including breast cancer, ovarian cancer, gynecological cancers and
 15 choriocarcinoma) as well as in brain, bone carcinoid, nasopharyngeal, retroperitoneal, thyroid and soft tissue tumours. It also finds application in the treatment or prophylaxis of cancers of unknown primary site.

20

XII. Posology

Those skilled in the art will be readily able to determine the amount of leukocyte composition to be autotransplanted in the medical applications according to the invention. It should be noted that as few as 0.01×10^8 (e.g. $1-10 \times 10^8$) mature
 25 lymphocytes (which can be derived from a single sample of approximately 100 ml of normal human blood) are sufficient to boost the immune system of a subject and hence may have a beneficial effect according to the autologous transplantation method of the invention. It should be noted that the removal of a unit of blood is commonplace with over three million units of blood being taken, for allografting,
 30 from individuals annually in the UK alone.

The leukocyte composition administered may be derived from a single blood sample, or may constitute a pool of leukocyte compositions derived from a plurality of different blood samples taken from a donor individual at different

times. The leukocyte composition administered may constitute all or a fraction of the deposited material, but preferably constitutes only a fraction thereof in order that multiple dosing can be achieved, optionally following cellular expansion of the residue (for example, T cell numbers may be increased by *in vitro* expansion using standard methods).

In applications based on T-cell activity, the number of mature T-cells administered is at least 0.01×10^8 , more preferably at least 0.1×10^8 , more preferably at least 1×10^8 (e.g. at least $1-10 \times 10^8$). The preferred ranges are 0.01×10^8 to 10^{10} mature T lymphocytes, such as 0.1×10^8 to 10^{10} , 1×10^8 to 10^{10} or 1×10^9 to 10^{10} mature T lymphocytes.

Thus, the mature T-cell sample acquired for autotransplantation is at least 0.01×10^8 , generally in the range of $10^8 - 10^{10}$ CD3⁺ mature T-cells, preferably $2 \times 10^8 - 10^{10}$, more preferably $3 \times 10^8 - 10^{10}$ CD3⁺ and even more preferably $4-5 \times 10^8 - 10^{10}$ CD3⁺ mature T-cells.

Conveniently, each sample prepared for autotransplantation contains 3×10^8 CD3⁺ mature T-cells, more preferably 5×10^8 and even more preferably 1×10^9 CD3⁺ mature T-cells. If sufficient resources of blood are available from an individual, even more preferably still $4-5 \times 10^9$ CD3⁺ mature T-cells or 10^{10} CD3⁺ mature T-cells may be used.

Preferably, the mature T-cell subpopulation sample acquired for autotransplantation which is CD3⁺ and CD8⁺ is at least 0.01×10^8 , generally in the range of $0.25 \times 10^8 - 0.25 \times 10^{10}$, and more preferably $0.5 \times 10^8 - 0.25 \times 10^{10}$, and even more preferably $0.75 \times 10^8 - 0.25 \times 10^{10}$, and even more preferably still $0.75 \times 10^8 - 0.25 \times 10^{10}$ or $1.00 - 1.25 \times 10^8 - 0.25 \times 10^{10}$. Specific CD3⁺ and CD8⁺ cell numbers in each sample prepared for grafting is conveniently of the order of 0.2×10^8 , preferably 0.4×10^8 , or more preferably 1×10^8 , or still more preferably 2×10^8 , or more preferably 3×10^8 , or more preferably 5×10^8 . If sufficient resources from an individual are available, 1×10^9 , preferably 2×10^9 , 4×10^9 , or more preferably 1×10^{10} CD3⁺ and CD8⁺ cells may be used.

Preferably, the mature T-cell subpopulation sample acquired for autologous transplantation which is CD3⁺ and CD4⁺ is at least 0.01×10^8 , generally in the range of $0.1 \times 10^8 - 0.5 \times 10^{10}$, and more preferably $0.65 \times 10^8 - 0.5 \times 10^{10}$, and even more preferably $0.85 \times 10^8 - 0.5 \times 10^{10}$, and even more preferably still $1 \times 10^8 - 0.5 \times 10^{10}$ or $1.8 - 3.6 \times 10^8 - 0.5 \times 10^{10}$. Specific CD3⁺ and CD4⁺ cell numbers in each sample prepared for grafting is conveniently of the order of 0.2×10^{10} , preferably 0.3×10^8 , or more preferably 0.4×10^8 , 0.5×10^8 , 1×10^8 , 2×10^8 , 3×10^8 , 4×10^8 , or more preferably 5×10^8 . If sufficient resources from an individual are available, 1×10^9 , or more preferably 2×10^9 , or more preferably 1×10^{10} CD3⁺ and CD4⁺ cells may be used.

Preferably, the mature T-cell natural killer subpopulation sample acquired for autotransplantation which is CD3⁺ and CD16/56⁺ is at least 0.01×10^8 , generally in the range of $0.01 \times 10^8 - 0.5 \times 10^{10}$, preferably $0.02 \times 10^8 - 0.5 \times 10^{10}$, more preferably $0.03 \times 10^8 - 0.5 \times 10^{10}$, and even more preferably still $0.5 \times 10^8 - 0.5 \times 10^{10}$ or $0.5 - 2 \times 10^8$ to 0.5×10^{10} . Specific CD3⁺ and CD16/56⁺ cell numbers in each sample prepared for grafting is conveniently of the order of 0.01×10^8 , 0.2×10^8 , 0.3×10^8 , 0.5×10^8 , 1×10^8 , 2×10^8 , 3×10^8 , 5×10^8 , or more preferably, if sufficient resources are available, 1×10^9 , or more preferably 2×10^9 , or more preferably 1×10^{10} CD3⁺ and CD16/56⁺ cells may be used.

In addition, the mature lymphocyte cell sample may preferably include B cells, such as CD19⁺ B lymphocytes. The mature B-cell sample included in the T-cell sample may be at least 10^7 , 10^8 or 10^9 , generally in the range of $10^7 - 10^{10}$ mature B-cells and preferably $2 \times 10^7 - 10^{10}$ mature B-cells, more preferably $3 \times 10^7 - 10^{10}$ mature B-cells, and even more preferably $4 - 5 \times 10^7 - 10^{10}$ mature B-cells.

Specific numbers of B-cells in autograft is conveniently of the order of 3×10^7 , preferably 5×10^8 , more preferably 1×10^8 mature B-cells, and even more preferably still $4 - 5 \times 10^9$ or 10^{10} mature B-cells.

In addition, the lymphocyte cell sample may preferably include dendritic cells. The dendritic cell sample may be at least 10^7 , 10^8 or 10^9 in number, and generally in the range of $10^7 - 10^{10}$ dendritic cells and preferably $2 \times 10^7 - 10^{10}$ cells, more

preferably $3 \times 10^7 - 10^{10}$ cells, and even more preferably $4-5 \times 10^7 - 10^{10}$ cells.

Specific numbers of dendritic cells in an autograft is conveniently of the order of 3×10^7 , preferably 5×10^8 , more preferably 1×10^8 , and even more preferably still
5 $4-5 \times 10^9$ or 10^{10} mature B-cells.

XIII. Equivalents

10 The foregoing description details presently preferred embodiments of the present invention which are therefore to be considered in all respects as illustrative and not restrictive. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents, modifications and variations to the specific embodiments of the invention described specifically
15 herein. Such equivalents, modifications and variations are intended to be (or are) encompassed in the scope of the following claims.

CLAIMS:

1. A process for producing a leukocyte bank suitable for CAT therapy comprising the steps of:

- 5 (a) providing a blood sample from a healthy donor individual;
 (b) selectively separating and collecting leukocytes from the sample; and
 (c) selectively separating and collecting red blood cells and/or platelets and/or
 plasma from the sample; and
10 (d) cryogenically preserving the leukocytes (and optionally the red blood cells
 and/or platelets and/or plasma);

wherein steps (b) and (c) are conducted with an automated leukapheresis device comprising a separation device (e.g. a centrifuge rotor or filter), a leukapheresis tubing set and one or more pumps for conveying the sample through the tubing set and the separated leukocytes into a collection vessel.

15

2. The process of claim 1 wherein the separated leukocytes are collected in a holding vessel.

3. The process of claim 2 further comprising the step of transferring two or more
20 aliquots of the separated leukocytes collected in the holding vessel to two or more independent storage vessels, optionally wherein each of the independent storage vessels comprises a cryopreservation medium in fluid communication therewith.

4. The process of claim 3 wherein the storage vessels are double containment
25 storage vessels.

5. The process of any one of claims 3 to 4 further comprising the step of mixing each aliquot of the separated leukocytes with a cryopreservation medium within each storage vessel.

30

6. The process of any one of the preceding claims further comprising the step of retrievably depositing the preserved leukocytes into two or more independent storage systems to produce a leukocyte bank which exhibits deposit redundancy.

7. The process of any one of the preceding claims wherein the separation and collection steps are conducted within a closed or functionally closed system.

5 8. The process of any one of the preceding claims wherein the separation and collection steps are applied iteratively to a series of blood samples from different healthy donor individuals.

9. The process of any one of the preceding claims further comprising the step of digitally storing information obtained from each donor individual in a digital
10 information unit so as to permit matching of leukocyte deposit and donor for later autologous transplantation.

10. The process of any one of the preceding claims wherein the cryogenic preservation step (d) comprises freezing to a temperature at or below about -
15 160°C.

11. The process of any one of claims 1 to 9 wherein the cryogenic preservation step (d) comprises freezing to a temperature at or below about -269°C.

20 12. The process of any one of claims 3 to 11 wherein the cryopropreservation medium comprises a penetrating cryoprotectant.

13. The process of claim 12 wherein the penetrating cryoprotectant comprises DMSO.

25 14. The process of claim 13 wherein the DMSO is present at a concentration of up to 10%.

15. The process of any one of claims 3 to 14 wherein cryopreservation medium
30 further comprises an anticoagulant.

16. The process of claim 15 wherein the anticoagulant comprises an anticoagulant selected from acid citrate dextrose, EDTA and heparin.

17. The process of any one of claims 3 to 16 wherein the cryopreservation medium further comprises a nuclease.
18. The process of claim 17 wherein the nuclease comprises ribonuclease and/or deoxyribonuclease.
19. The process of any one of claims 3 to 18 wherein the cryopreservation medium further comprises a physiologically acceptable medium.
20. The process of claim 19 wherein the physiologically acceptable medium is phosphate buffered saline.
21. The process of any one of claims 3 to 20 wherein the cryopreservation medium further comprises a proteinaceous and/or sugar and/or polysaccharide composition.
22. The process of claim 21 wherein the proteinaceous composition comprises blood serum or a blood serum component.
23. The process of claim 22 wherein the proteinaceous composition comprises blood albumin (e.g. bovine serum albumin or human serum albumin).
24. The process of claim 23 wherein the proteinaceous composition comprises autologous blood serum separated and collected in step (c).
25. The process of any one of the preceding claims wherein the healthy donor individual:
- (a) is predisposed to a leukocyte deficiency; and/or
 - (b) is not in remission from a leukocyte deficiency; and/or
 - (c) is juvenile, adolescent or adult; and/or
 - (d) is at risk of developing a leukocyte deficiency; and/or
 - (e) is a human individual between the ages of about 12 to 30 (e.g. 15 to 25); and/or
 - (f) has a fully-developed immune system.

26. The process of any one of the preceding claims wherein the blood sample is an isolated blood sample.

5 27. The process of claim 26 wherein the isolated blood sample has a volume of 450 to 500ml.

28. The process of any one of the preceding claims wherein steps (b) to (d) are applied iteratively to a series of blood samples from the same healthy donor
10 individual.

29. The process of claim 28 wherein steps (b) to (d) are applied to 2-12 samples taken from the same healthy donor individual over the course of one year.

15 30. The process of any one of the preceding claims wherein the leukocytes separated in step (b) comprise (or consist essentially of):

- (a) granulocytes; and/or
- (b) lymphocytes; and/or
- (c) monocytes.

20

31. The process of claim 30 wherein the separation step (b) comprises the selective separation of a particular class or type of leukocyte.

32. The process of claim 31 wherein the separation step (b) comprises the
25 selective separation of B-cells and/or T-cells and/or dendritic cells and/or mixtures thereof.

33. The process of any one of claims 9 to 32 wherein the information stored comprises:

- 30
- (a) genetic information; and/or
 - (b) the date at which the blood sample was collected from the donor individual; and/or
 - (c) the age and sex of the donor individual; and/or
 - (d) the clinical status of the donor individual; and/or

- (e) a medical history of the donor individual; and/or
- (f) biographical data identifying the donor individual; and/or
- (g) details of the processing and storage conditions used; and/or
- (h) data identifying the person(s) responsible for processing the sample(s).

5

34. The process of claim 33 wherein the information comprises genetic information selected from:

- (a) sequence information relating to one or more gene(s); and/or
- (b) single nucleotide polymorphism (SNP) data; and/or
- 10 (c) a genetic fingerprint.

35. The process of any one of claims 9 to 34 wherein the digital information unit comprises at least one digital computer.

15 36. The process of any one of claims 3 to 35 further comprising the step of labelling the storage vessels with information sufficient to permit matching of the leukocyte deposit and donor.

37. The process of claim 36 wherein the information:

- 20 (a) describes the contents of the vessel (for example, sample size, number and/or volume); and/or
- (b) identifies the leukocyte bank; and/or
- (c) records the date at which the blood sample was collected from the donor individual; and/or
- 25 (d) comprises a statement that each package is for single patient use only; and/or
- (e) comprises instructions for opening, aseptic presentation and further storage.

30 38. The process of claim 36 or 37 wherein the labelling comprises physical attachment of a bar code to the storage vessels.

39. The process of any one of the preceding claims wherein the leukocytes are treated:

- (a) *in vivo* prior to provision of the blood sample;
- (b) *in vitro* prior to separation step (b);
- (c) *in vitro* after separation step (b) but prior to preservation step (d);
- (d) *in vitro* after preservation step (d).

5

40. The process of any one of the preceding claims wherein the leukocytes are selectively separated and or collected from the sample by:

- (a) isolated leukapheresis;
- (b) continuous or interrupted flow centrifugation leukapheresis;
- 10 (c) continuous or interrupted flow filtration leukapheresis.

41. A process for producing a leukocyte composition suitable for CAT therapy comprising the steps of:

- (e) producing a leukocyte cell bank by the process of any one of the preceding
- 15 claims;
- (f) matching the donor individual with a leukocyte deposit to identify an autologous leukocyte deposit (e.g. using the information as defined in any one of claims 9 to 40);
- (g) retrieving a storage vessel containing an aliquot of preserved autologous
- 20 leukocytes; and
- (h) revitalizing the preserved autologous leukocytes to produce a leukocyte composition for autotransplantation into the donor individual.

42. The process of claim 41 wherein the leukocytes are revitalized by thawing

25 and/or dilution.

43. The process of claim 41 or claim 42 for producing a leukocyte composition for restorative autotransplantation.

30 44. A leukocyte bank obtainable (or obtained) by the process of any one of claims 1 to 40.

45. A leukocyte composition obtainable (or obtained) by the process of any one of claims 41 to 43.

46. The leukocyte composition of claim 45 for use in therapy or prophylaxis (e.g. for CAT therapy).
- 5 47. Use of the leukocyte composition of claim 45 for the manufacture of a medicament for use in autotransplantation (e.g. in restorative autotransplantation).

PCT/GB2004/004173



**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.